REMARKS

Status of Claims

The amendments to claims 13 and 51 are supported by claims 44 and 56, respectively. Claims 44 and 56 have been canceled. No new matter has been added.

Upon entry of this amendment, claims 13-16, 43, 46, 47, 51-55 and 57-62, are present and active in the application.

Request for Reconsideration

Applicants thank Examiner Huynh for the courteous and helpful discuss held with applicants' representative. During this discussion, Examiner Huynh clarified the meaning of "disrupting" as including any method of allowing antibodies to enter apoptotic bodies. Applicants have clarified the claims accordingly.

Above-average levels of apoptotic bodies in the bloodstream have been correlated with the presence of tumors and cancers in a subject. While this statement appears to contradict the general observation that apoptotic levels are decreased in tumor and cancer cells, the statement is not absolute. Resistance to apoptosis is usually a late event in malignant progression--that is, resistance to apoptosis increases as the cancer grows and becomes metastatic. Therefore, early stage tumors can be characterized by slow overall growth, reflecting a high proliferation rate balanced by a high level of apoptosis. Even in late stage tumors with relatively low rates of apoptosis, the absolute number of apoptotic bodies can be high due to the large tumor mass.

Nucleolin and PARP-1 have been discovered to be unexpectedly convenient and reliable markers for the detection of apoptotic bodies, especially those shed into circulation. Detecting these antigens in circulation, such as in plasma or serum, correlates with levels of apoptosis that overwhelm the usual apoptotic body-clearing systems, such as macrophages and/or neighboring cells to the site of apoptosis.

Normal, healthy subjects have undetectable levels of apoptotic bodies in the circulation, because the usual apoptotic body-clearing mechanisms would remove them before they accumulate to detectable levels. Consequently, nucleolin and PARP-1 are undetectable in the circulation of healthy subjects. The detection of nucleolin or

PARP-1 in the circulation means that high levels of these proteins are present in the circulation, which correlates with excessive apoptosis.

The invention as now claimed is directed to two methods for detecting excessive apoptosis in a blood sample from a subject. One method includes reacting an antibody that binds specifically to nucleolin, to detect apoptotic bodies in the blood sample, wherein detecting high levels of nucleolin correlates with excessive apoptosis. The second method includes reacting an antibody that binds specifically to poly(ADP-ribose) polymerase (PARP-1), to detect apoptotic bodies in the blood sample, wherein detecting high levels of PARP-1 correlates with excessive apoptosis.

The rejections of the claims under 35 U.S.C. § 103 over Holdenrieder et al. in view Martelli et al., and optionally further in view Hanakhi et al., Solani et al., Gougeon et al., Andrade et al., and/or Aihara et al., are respectfully traversed. Neither Holdenrieder et al. nor Martelli et al. provide any guidance as to the presence or absence of either nucleolin or PARP-1 in apoptotic bodies. Neither Holdenrieder et al. nor Martelli et al. correlate the presence of either nucleolin or PARP-1 in a blood sample with excessive apoptosis.

Holdenrieder et al. describes nucleosomes in serum of patients with benign and malignant diseases. In the abstract, this references postulates that "the concentration of nucleosomes in serum *might* be a useful tool for monitoring the biochemical response during antitumor therapy, especially for the early estimation of therapeutic efficacy" (emphasis added). The authors note two types of cell death: "Whereas cells in the center of solid tumors mainly die via oncosis (formerly known as necrosis), cells at the margins are preferentially eliminated by apoptosis" (column 1, page 114; citations omitted). Further noted is that nucleosomes "are complexes formed from DNA and histones", and

> Under physiological conditions, these nucleosomes are packed into apoptotic bodies and engulfed by macrophages and neighboring cells. However, at high rates of apoptosis, these phagocytosing mechanisms are saturated, leading to elevated concentrations of nucleosomes in the circulating blood.
>
> (column 1, page 114; citations omitted).

Ultimately, Holdenrieder et al. conclude that although "the nucleosomes in serum [they] measured derived at least partly from the apoptotic death of tumor cells", this could not be used to detect excessive apoptosis, because in "addition to apoptosis, oncosis or a mixture of both types of cell death could lead to an increase of the concentration of nucleosomes in serum" (bottom of column 1, page 118; citations omitted). Holdenrieder et al. is silent regarding both nucleolin and PARP-1. Holdenrieder et al. does not provide any guidance as to the presence or absence of either nucleolin or PARP-1 in apoptotic bodies. Holdenrieder et al. does not correlate the presence of either nucleolin or PARP-1 in a blood sample with excessive apoptosis.

Martelli et al. concerns the intracellular distribution of the nucleolar protein components during the apoptosis process in camptothecin-treated HL60 cells. Martelli et al. only examines cells (apoptotic cells), never apoptotic bodies. Martelli et al. does not provide any guidance as to the presence or absence of either nucleolin or PARP-1 in apoptotic bodies. Martelli et al. does not correlate the presence of either nucleolin or PARP-1 in a blood sample with excessive apoptosis.

Hanakhi et al., Solani et al., Gougeon et al., Andrade et al., and Aihara et al. have only been cited for elements of dependent claims. These references do not provide any guidance as to the presence or absence of either nucleolin or PARP-1 in apoptotic bodies. These references do not correlate the presence of either nucleolin or PARP-1 in a blood sample with excessive apoptosis.

The claimed invention includes reacting an antibody that binds specifically to nucleolin, or reacting an antibody that binds specifically to poly(ADP-ribose) polymerase (PARP-1), to detect apoptotic bodies in the blood sample, wherein detecting high levels of nucleolin or PARP-1 correlates with excessive apoptosis. The applied references do not provide any guidance as to the presence or absence of either nucleolin or PARP-1 in apoptotic bodies. The applied references do not correlate the presence of either nucleolin or PARP-1 in a blood sample with excessive apoptosis. Accordingly, the claimed invention is not obvious over the applied references. Withdrawal of these grounds of rejection is respectfully requested.

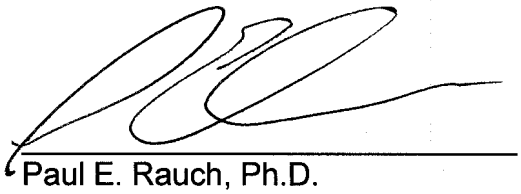
The rejection of the claims under 35 U.S.C. § 112, first paragraph, has been obviated by appropriate amendment.

The objection to claim 14 is respectfully traversed. The terms "tumor" and "cancer" are not redundant, since some tumors are not cancerous, and some cancers do not form tumors. Withdrawal of this rejection is respectfully requested.

The objection to claims 15 and 53 is respectfully traversed. These claims specify only one member of the Markush group listed in claims 14 and 52, respectively, and therefore the dependency of these claims on the same independent claim is appropriate. However, if the Examiner insists that the current dependency is improper, applicants will change the dependency of claims 15 and 53.

Applicants submit that the application is now in condition for allowance. Applicants encourage Dr. Huynh to contact the undersigned by telephone to discuss subject matter that may be allowable following entry of this amendment or by way of entry of an Examiner's amendment. Early notice of such action is earnestly solicited.

Respectfully submitted,



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